EUCAST Technical Note on *Aspergillus* and amphotericin B, itraconazole, and posaconazole

M. C. Arendrup[1], M. Cuenca-Estrella[2], C. Lass-Flörl[3], W. W. Hope[4] and The European Committee on Antimicrobial Susceptibility Testing Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST)*

1) *Unit of Mycology and Parasitology, Department of Microbiological Surveillance and Research, Statens Serum Institute, Copenhagen, Denmark,* 2) *Servicio de Micología, Instituto de Salud Carlos III, Madrid, Spain,* 3) *Hygiene and Medical Microbiology, Innsbruck Medical University, Innsbruck, Austria and* 4) *Translational Medicine, University of Manchester, Manchester, UK*

Abstract

The European Committee on Antimicrobial Susceptibility Testing Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST) has determined breakpoints for amphotericin B, itraconazole and posaconazole for *Aspergillus* species. This Technical Note is based on the EUCAST amphotericin B, itraconazole and posaconazole rationale documents (available on the EUCAST website: http://www.eucast.org/antifungal_susceptibility_testing_afst/rationale_documents_for_antifungals/). The amphotericin B and itraconazole breakpoints are based on epidemiological cut-off values and clinical experience. The posaconazole breakpoints are also based on pharmacokinetic and pharmacodynamic data. Breakpoints will be reviewed regularly or when new data emerge.

Keywords: Amphotericin B, *Aspergillus*, breakpoints, EUCAST Technical Note, itraconazole, posaconazole, susceptibility testing

Original Submission: 23 March 2012; **Accepted:** 31 March 2012
Editor: E. Roilides
Article published online: 5 April 2012
Clin Microbiol Infect 2012; **18:** E248–E250
10.1111/j.1469-0691.2012.03890.x

Corresponding author: M. C. Arendrup, Unit of Mycology, Department of Microbiological Surveillance and Research, Statens Serum Institute, Ørestads Boulevard 5, DK-2300 Copenhagen, Denmark
E-mail: maca@ssi.dk or maiken@arendrup.dk

*EUCAST-AFST: M. C. Arendrup (Chairman, Denmark), W. W. Hope (Secretary), C. Lass-Flörl, Steering Committee (Austria), M. Cuenca-Estrella, Steering Committee (Spain), S. Arikan (Turkey), F. Barchiesi (Italy), J. Bille (Switzerland), E. Chryssanthou (Sweden), P. Gaustad (Norway), A. Groll (Germany), H. Järv (Estonia), N. Klimko (Russia), O. Lortholary, (France), C. Moore (UK), A. Velegraki (Greece), and P. Verweij (The Netherlands).

The European Committee on Antimicrobial Susceptibility Testing Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST) has determined breakpoints for amphotericin B, itraconazole and posaconazole against *Aspergillus* species. This Technical Note is based on the respective EUCAST rationale documents (available at: http://www.eucast.org/antifungal_susceptibility_testing_afst/rationale_documents_for_antifungals/). The rationale documents include more detail and published references related to the selection of EUCAST-AFST breakpoints.

Amphotericin B is a polyene antifungal agent with broad-spectrum activity. A number of different formulations are available in Europe: amphotericin B deoxycholate (DAmB), liposomal amphotericin B (LAmB), amphotericin B lipid complex (ABLC), and amphotericin B colloidal dispersion (ABCD). These compounds have distinct pharmacokinetic and toxicity profiles. DAmB and LAmB are licensed for first-line treatment of invasive aspergillosis. ABLC and ABCD are licensed as second-line agents. Licensed dosages are as follows: DAmB, 1–1.5 mg/kg; LAmB, 3–10 mg/kg; ABLC, 3–5 mg/kg; and ABCD, 3–5 mg/kg. The epidemiological cut-off values (ECOFFs) were established with the use of MIC values from many sources (Table 1). There are no known resistance mechanisms in *Aspergillus fumigatus* that result in reduced susceptibility to amphotericin B. Multiple clinical studies have suggested that amphotericin B is active against the wild-type population of *Aspergillus* species (with the exception of *Aspergillus terreus*). A direct correlation between the MIC of amphotericin B and clinical outcomes is not possible, because EUCAST methodology has not been used; invasive isolates have not been recovered or have not been identified to the species level. Furthermore, there are no preclinical pharmacokinetic–pharmacodynamic studies that can be used to support the setting of breakpoints. Both preclinical and clinical studies suggest that *A. terreus* is a poor target for amphotericin B, and can therefore be reasonably reported as resistant without further testing [1]. Breakpoints were established with the use of both microbiological, pharmacokinetic and clinical data.

Itraconazole is a triazole antifungal agent that can be used for the treatment of both invasive and allergic syndromes caused by *Aspergillus* species. Available formulations vary across Europe, but include capsules and a cyclodextrin suspension that is suitable for both oral and intravenous administration. The absorption of itraconazole capsules is increased by food and acidic gastric conditions. The oral suspension has a better oral bioavailability that is not affected by gastric acidity. The licensed dosage is 200–400 mg/day. Therapeutic drug monitoring is frequently recommended to ensure adequate systemic drug exposure [2–6]. ECOFFs were established with the use of MICs from many sources,

TABLE 1. ***Aspergillus*** **species-specific amphotericin B, itraconazole and posaconazole EUCAST epidemiological cut-off values (ECOFFs) and breakpoints**

	Species-related breakpoints[b] (mg/L)								
	Amphotericin B			Itraconazole			Posaconazole[c]		
Species[a]	ECOFF	Breakpoint		ECOFF	Breakpoint		ECOFF	Breakpoint	
A. flavus	4	IE[d]	IE[d]	1	S ≤ 1	R > 2	0.5	IE[d]	IE[d]
A. fumigatus	1	S ≤ 1	R > 2	1	S ≤ 1	R > 2	0.25	S ≤ 0.12	R > 0.25
A. nidulans	ND	Note[e]	Note[e]	1	S ≤ 1	R > 2	0.5	IE[d]	IE[d]
A. niger	1	S ≤ 1	R > 2	4	IE[d]	IE[d]	0.5	IE[d]	IE[d]
A. terreus	4	–	–	1	S ≤ 1	R > 2	0.25	Note[e]	Note[e]
A. versicolor	ND	ND	ND	ND	IE[d]	IE[d]	ND	Nd	ND

IE, insufficient evidence; ND, not determined, owing to insufficient data.
'–' indicates that susceptibility testing is not recommended, as the species is a poor target for therapy with the drug. Isolates may be reported as R without prior testing.
[a]There is insufficient evidence to set non-species-related breakpoints.
[b]In order to simplify the EUCAST tables, the intermediate category is not listed. It is readily interpreted as the value between the S and the R breakpoints. For example, for MIC breakpoints listed as S ≤ 1 and R > 2, the intermediate category is 2 (technically >1–2). There is insufficient clinical evidence to set breakpoints for species other than those listed.
[c]Provided that adequate drug exposure has been confirmed by the use of therapeutic drug monitoring.
[d]The MIC values are, in general, higher than those for *A. fumigatus*. Whether this translates into a poorer clinical response is unknown. There is insufficient evidence to set breakpoints for these species.
[e]There is inadequate clinical information on the clinical outcome for patients infected with wild-type strains, although the MIC distributions are similar to those for *A. fumigatus*.

and are summarized in Table 1. Resistance to azoles has been detected in *A. fumigatus* isolates in many European countries over the last decade [7–12]. Most commonly, the resistance is linked to point mutations in the target gene *CYP51A*. However, other mechanisms may also play a role [13,14]. The itraconazole MICs for mutant isolates vary according to the underlying mechanism, but are typically ≥4 mg/L for the most commonly identified mutants [8]. Isolates with reduced susceptibility to itraconazole are frequently cross-resistant to other triazoles, and specific testing is recommended. Acquired resistance mechanisms within the other *Aspergillus* species are poorly understood. However, a recent study reported itraconazole MIC elevation in an *A. terreus* isolate with a *CYP51A* mutation, suggesting that species other than *A. fumigatus* may acquire triazole resistance [15]. Clinical studies suggest that itraconazole is active against wild-type populations of *Aspergillus* species. A direct correlation between the MIC and clinical outcome is not possible, because clinical isolates are often not identified to the species level, and susceptibility has not been determined with EUCAST methodology. There are no preclinical pharmacokinetic–pharmacodynamic data that can be used to provide decision support for setting itraconazole breakpoints. Breakpoints were established with the use of both microbiological, pharmacokinetic and clinical data.

Posaconazole is a broad-spectrum triazole antifungal agent approved for refractory or second-line invasive fungal diseases (including aspergillosis) and for prophylaxis of invasive fungal infections in immunocompromised patients. The licensed regimens for the prophylaxis and treatment of invasive disease are 200 mg every 8 h and 400 mg every 12 h, respectively. Oral bioavailability is highly variable. Therapeutic drug monitoring is increasingly recommended, although definitive targets are not known. Trough concentrations of 0.7 and 1 mg/L are reasonable for the prophylaxis and treatment of established disease, respectively [5,16,17]. The ECOFFs were established by the use of MIC values from many sources (Table 1). Reduced susceptibility to posaconazole is associated with mutations in *CYP51A* [8–12]. The MICs of *CYP51A* mutants are mechanism-dependent, but are typically ≥0.25 mg/L [8]. The MIC distribution of *CYP51A* mutants overlaps with that of the wild-type population, which is problematic for the setting of breakpoints [12]. Infections caused by these isolates should not be treated with posaconazole unless specific clinical evidence becomes available suggesting that such an approach is effective. Isolates resistant to posaconazole but susceptible to itraconazole are extremely rare. Therefore, itraconazole susceptibility testing may be helpful as an initial screening marker for the detection of multi-azole resistance. A direct correlation between MIC and clinical outcomes is not possible, because the relevant studies have not been performed. Near-maximal antifungal efficacy for posaconazole salvage therapy of invasive aspergillosis is observed with mean serum concentrations of *c.* 1.25 mg/L [18]. Pharmacodynamic targets can only be achieved for the entire wild-type population if adequate systemic drug exposure is confirmed with the use of therapeutic drug monitoring [19,20]. Because of overlapping MIC distributions for the wild-type and mutant populations, and because of the unacceptably low target attainment rates for isolates at the upper end of the wild-type population, the breakpoints are set one step lower than the ECOFF. This is a conservative decision, and will be revised if specific clinical and/or pharmacodynamic data arise suggesting that isolates currently classified

as 'I' or 'R' can be safely treated with posaconazole. Breakpoints were established by the use of microbiological, clinical and pharmacokinetic–pharmacodynamic data.

EUCAST breakpoints only apply to licensed regimens. The breakpoints will be reviewed when more data are available for *Aspergillus* species that were not assigned breakpoints during the present review, when there are clinical data for isolates with MIC values outside the wild-type distribution, or when there are further data related to optimal drug exposures.

Acknowledgements

W. W. Hope is supported by a National Institute of Health Clinician Scientist Award.

Transparency Declaration

The authors do not have any potential conflicts of interests related particularly to this paper. M. C. Arendrup: has received research grants and acted as speaker for Astellas, Gilead, MSD, and Pfizer, and been a consultant for Gilead, MSD, and Pcovery. M. Cuenca-Estrella: has received grant support from Astellas Pharma, bioMerieux, Gilead Sciences, Merck Sharp and Dohme, Pfizer, Schering Plough, Soria Melguizo SA, Ferrer International, the EU, the ALBAN program, the Spanish Agency for International Cooperation, the Spanish Ministry of Culture and Education, The Spanish Health Research Fund, The Instituto de Salud Carlos III, The Ramon Areces Foundation, and The Mutua Madrileña Foundation. He has been an advisor/consultant to the Panamerican Health Organization, Gilead Sciences, Merck Sharp and Dohme, Pfizer, and Schering Plough. He has been paid for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer, and Schering Plough. C. Lass-Flörl: has received research grants and honorarium as consultant and/or speaker for Pfizer, Astellas, Gilead and Merck. W. W. Hope: has received research grants and honorarium as consultant and/or speaker for Pfizer, Astellas, Gilead, Merck Vectura and F2G.

References

1. Lass-Florl C, Griff K, Mayr A *et al.* Epidemiology and outcome of infections due to *Aspergillus terreus*: 10-year single centre experience. *Br J Haematol* 2005; 131: 201–207.
2. Denning DW, Lee JY, Hostetler JS *et al.* NIAID Mycoses Study Group multicenter trial of oral itraconazole therapy for invasive aspergillosis. *Am J Med* 1994; 97: 135–144.
3. Glasmacher A, Hahn C, Leutner C *et al.* Breakthrough invasive fungal infections in neutropenic patients after prophylaxis with itraconazole. *Mycoses* 1999; 42: 443–451.
4. Lestner JM, Roberts SA, Moore CB, Howard SJ, Denning DW, Hope WW. Toxicodynamics of itraconazole: implications for therapeutic drug monitoring. *Clin Infect Dis* 2009; 49: 928–930.
5. Andes D, Pascual A, Marchetti O. Antifungal therapeutic drug monitoring: established and emerging indications. *Antimicrob Agents Chemother* 2009; 53: 24–34.
6. Boogaerts MA, Verhoef GE, Zachee P, Demuynck H, Verbist L, De Beule K. Antifungal prophylaxis with itraconazole in prolonged neutropenia: correlation with plasma levels. *Mycoses* 1989; 32 (suppl 1): 103–108.
7. Denning DW, Radford SA, Oakley KL, Hall L, Johnson EM, Warnock DW. Correlation between in-vitro susceptibility testing to itraconazole and in-vivo outcome of *Aspergillus fumigatus* infection. *J Antimicrob Chemother* 1997; 40: 401–414.
8. Howard SJ, Cerar D, Anderson MJ *et al.* Frequency and evolution of azole resistance in *Aspergillus fumigatus* associated with treatment failure. *Emerg Infect Dis* 2009; 15: 1068–1076.
9. Howard SJ, Arendrup MC. Acquired antifungal drug resistance in *Aspergillus fumigatus*: epidemiology and detection. *Med Mycol* 2011; 49 (suppl 1): 90–95.
10. Mortensen KL, Jensen RH, Johansen HK *et al. Aspergillus* species and other molds in respiratory samples from patients with cystic fibrosis: a laboratory-based study with focus on *Aspergillus fumigatus* azole resistance. *J Clin Microbiol* 2011; 49: 2243–2251.
11. Mellado E, Garcia-Effron G, Alcazar-Fuoli L *et al.* A new *Aspergillus fumigatus* resistance mechanism conferring in vitro cross-resistance to azole antifungals involves a combination of cyp51A alterations. *Antimicrob Agents Chemother* 2007; 51: 1897–1904.
12. Verweij PE, Howard SJ, Melchers WJ, Denning DW. Azole-resistance in *Aspergillus*: proposed nomenclature and breakpoints. *Drug Resist Updat* 2009; 12: 141–147.
13. Arendrup MC, Mavridou E, Mortensen KL *et al.* Development of azole resistance in *Aspergillus fumigatus* during azole therapy associated with change in virulence. *PLoS ONE* 2010; 5: e10080.
14. Bueid A, Howard SJ, Moore CB *et al.* Azole antifungal resistance in *Aspergillus fumigatus*: 2008 and 2009. *J Antimicrob Chemother* 2010; 65: 2116–2118.
15. Arendrup MC, Jensen RH, Grif K *et al. In vivo* emergence of *Aspergillus terreus* with reduced azole susceptibility and a *Cyp51a* M217I alteration. *J Infect Dis* 2012; in press.
16. Jang SH, Colangelo PM, Gobburu JV. Exposure-response of posaconazole used for prophylaxis against invasive fungal infections: evaluating the need to adjust doses based on drug concentrations in plasma. *Clin Pharmacol Ther* 2010; 88: 115–119.
17. Howard SJ, Felton TW, Gomez-Lopez A, Hope WW. Posaconazole: the case for therapeutic drug monitoring. *Ther Drug Monit* 2012; 34: 72–76.
18. Walsh TJ, Raad I, Patterson TF *et al.* Treatment of invasive aspergillosis with posaconazole in patients who are refractory to or intolerant of conventional therapy: an externally controlled trial. *Clin Infect Dis* 2007; 44: 2–12.
19. Howard SJ, Lestner JM, Sharp A *et al.* Pharmacokinetics and pharmacodynamics of posaconazole for invasive pulmonary aspergillosis: clinical implications for antifungal therapy. *J Infect Dis* 2011; 203: 1324–1332.
20. Mavridou E, Bruggemann RJ, Melchers WJ, Mouton JW, Verweij PE. Efficacy of posaconazole against three clinical *Aspergillus fumigatus* isolates with mutations in the cyp51A gene. *Antimicrob Agents Chemother* 2010; 54: 860–865.